chain kinase (MLCK). Further geometric constraints regulate the nuclear to cytoplasmic ratio of HDAC3, a histone-deacetylase enzyme. Taken together, our work suggests that cellular geometric cues regulate chromatin remodeling processes via modulating acto-myosin contractility and nuclear-cytoplasmic shuttling of histone deceatylase enzymes.

2433-Pos Board B203
Impact of Nuclear Morphology on Gene Expression during Cellular Differentiation and Development
Abhishek Kumar.
MechanoBiology Institute (MBI), Singapore, Singapore.
Morphogenetic movements elicit differential gene expression programs during developmental processes, but the spatio-temporal evolution of nuclear morphology and its impact on genome function for lineage specificity is still unclear. Our results suggest that there exist an inter-play between nuclear morphology, inter-cellular connections and gene expression during the early development of *Drosophila* embryo. Quantitative morphometric analysis during development revealed the coupling between acto-myosin assembly and the emergence of nuclear shape. Physical perturbation to groups of cells in the developing embryo, using magnetic traps, alters nuclear morphology and induces defects in morphogenetic movement. As a consequence, these defects result in an altered segmental gene expression pattern. Our results highlight the importance of the emergence of prestressed nuclear morphology to genome regulatory processes.

2434-Pos Board B204
The Effect of Nucleosome Stability on Gene Expression Level
Rasesh Y. Parikh.
Georgia Institute of Technology, Atlanta, GA, USA.
Nucleosomes, which are the basic packaging units of chromatin, are stably positioned in promoters upstream of most stress-induced genes. These promoter nucleosomes are generally thought to repress gene expression due to exclusion; they prevent transcription factors from accessing their binding sites on the DNA. Some promoter nucleosomes, however, do not directly occlude transcription factor binding sites, and therefore their role in gene expression remains unknown. To understand the non-exclusive role of nucleosomes in gene expression, we designed a model promoter in budding yeast where a nucleosome intervenes between a transcription factor binding site in nucleosome depleted region and the transcription start site. To vary nucleosome stability, we constructed promoter variants with several different GC contents in the nucleosomal DNA sequence. We then measured the downstream gene expression level from these promoter variants at different induction levels using a fluorescent protein reporter. Our preliminary data show that relatively high nucleosome stability does not always correlate with relatively low gene expression level. This result suggests that nucleosomes might contribute to gene expression in a cooperative manner either by bringing otherwise distant DNA sites close together or by anchoring the SWI/SNF chromatin remodeling complex for efficient removal of adjacent nucleosomes.

2435-Pos Board B205
Structural Transitions in Higher Order Chromatin Assembly and Nuclear Plasticity during Stem Cell Differentiation
Shefali Talwar[1,2], G.V. Shivashankar[1].
[1]Mechanobiology Institute, NUS, Singapore, Singapore, [2]National Centre for Biological Sciences, Bangalore, India.
Undifferentiated cells integrate physico-chemical cues from the local microenvironment to elicit lineage specific gene expression programs. However the underlying mechanisms of this physical plasticity and how it impinges on gene expression is still unclear. In this study, using high resolution live-cell fluorescence polarization imaging, we analyze the spatio-temporal aspects of nuclear organization and chromatin structure in mouse embryonic stem (ES) cells and contrast them to a primary embryonic fibroblasts (PMEF). Higher-order chromatin compaction states exhibit unique features in ES cells, marked by homogeneous chromatin compaction but heterogeneity at the population level, but PMEFs evidence an inverse correlation. In addition, the nuclear lamina and actin cytoskeleton is highly flexible in ES cells but are frozen in PMEFs. This transition in nuclear architecture resembles that of fluid-like to solid-like transitions. Further the temporal evolution of nuclear plasticity is studied by differentiating ES cells on gelatin coated dishes. Taken together these results suggest that ES cells exhibit a broad epigenetic free energy landscape transitioning into a frozen configuration in higher-order chromatin assembly as lineage specific gene expression programs emerge.

2436-Pos Board B206
Single-Molecule Studies of the Bacterial Segregosome Component Spo0J
Thomas G.W. Graham.
Harvard University, Cambridge, MA, USA.
The ParAB system is a broadly conserved module involved in bacterial plasmid partitioning and chromosome segregation. The core DNA binding component of the system, ParB, binds to consensus parS sites in plasmid or chromosomal DNA but also interacts with adjacent DNA non-sequence-specifically. We have used single molecule techniques, including a new ''DNA motion capture'' assay, to characterize the interactions of the Bacillus subtilis ParB homolog Spo0J with double-stranded DNA. In contrast to previous models of ParB polymerization on DNA to form a filament, our results suggest that Spo0J forms higher-order complexes by trapping DNA loops.

2437-Pos Board B207
3D Organization of the Interphase Nucleus using Soft X Ray Tomography
Bertrand P. Cinquin, Rosanne Boudreau, Mark Legros, Carolyn Larabell.
USCF/LBL/ALS/NCXT, Berkeley, CA, USA.
The nucleus, although it has been studied for many decades, remains the body of many mysteries. In eukaryotic cells, DNA is found in two coexistent readable forms, an open, gene-rich region (euchromatin) that allows transcription of DNA into RNA, and a compacted region (heterochromatin) that contains silenced genes. In response to numerous extracellular stimuli, the cell must express specific proteins, which means that the DNA coding for these proteins must be in a transcriptionally active form. Consequently the balance between euchromatin and heterochromatin must be regulated during cell differentiation. Understanding this regulation requires fine-tuned biochemical and biophysical analyses and cutting-edge imaging techniques. Thanks to the soft X-ray microscope of the National Center for X-ray Tomography (Uchida et al., 2010; Larabell et al. 2010), high-resolution images of the 3D organization of the nucleus in the native state are now achievable. Cryo-immobilization of the cells (fast freezing) for x-ray imaging avoids chemical fixation artifacts associated with TEM. Imaging with X-rays in the water window energy range allows a natural contrast between water and biomolecules. X-ray imaging avoids perturbations caused by chemicals and osmotic changes associated with dehydration required for TEM, which cause deformation of nuclear structures (Finan and Guilack, 2010). As a result, x-ray tomography yields the first 3D views of nuclear organization in intact cells with a resolution of 50 nm. Our study indicates that heterochromatin forms a continuous network in 3D space, with no evidence of the chromatin patches described in TEM. Likewise, euchromatin regions are continuous. In addition distinct 3D chromatin patterns are linked to the differentiation/maturation state of the cell. Studying the 3D pattern of the nucleus using soft x-ray tomography is shedding new light on our understanding of cell differentiation.

2438-Pos Board B208
Ultrastructural Probes of Clusters of Open Regulatory Elements (Core) Within Chromatin
John H. Frenster, Jeannette A. Hovsepian.
Stanford University, Atherton, CA, USA.
Introduction:
Mammalian cells during interphase display clustering of DNase I-sensitive sites within the 10 nm micro-fibrils of active euchromatin (Song L, et al, Genome Research, August 19, 2011). Over 870,000 distinct CORE sites were identified as poised or active in gene transcription across an analysis of seven human cell lines, (*ibid*).
Methods:
We have developed a high-resolution electron microscopic technique for detecting DNase I-sensitive sites within intact single human cells. All such sites are confined to the euchromatin portion of the cell nucleus, and can be analyzed for location, number, shape, and size within each single cell analyzed within native biopsied tissue. Such ultrastructural probes can detect sites greater than 10 nm in diameter, and offer a global 3D view in 10 nm thick serial sections of the probed tissue after instant fixation during biopsy.